Volume 284, number 2, 257–262 FEBS 09846 June 1991

ADONIS 0014579391005561

Nucleotide sequences of the continuous and separated petA, petB and petD chloroplast genes in *Chlamydomonas reinhardtii*

S. Büschlen, Y. Choquet, R. Kuras and F.-A. Wollman

Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, 75005 Paris, France

Received 18 April 1991

We have mapped and sequenced the petA (cytf), petB (cytb6) and petD (subunit IV) genes on the chloroplast genome of *Chlamydomonas reinhardtii*. At variance with the pet genes in higher plant chloroplasts, the petB and petD genes are continuous, not adjacent and not located next to the psbB gene. The corresponding polypeptide sequences are highly conserved when compared with their counterparts from other sources but have a few features specific of algal cytb6/f complexes. In particular the transit sequence of cytf displays unique characteristics when compared with those previously described for cytf in higher plants.

Pet gene; Cytochrome b6/f complex; Transit peptide; *Chlamydomonas reinhardtii*

1. INTRODUCTION

The unicellular green alga *Chlamydomonas reinhardtii* has proven particularly useful in studying the genetic expression in eucaryotic cells performing oxygenic photosynthesis. Besides the opportunity to isolate and characterize photosynthesis mutants by conventional mutagenesis [1], it is now possible to perform site-directed mutagenesis using various transformation strategies [2]. Both approaches have been used extensively in the study of some of the major protein complexes of the photosynthetic apparatus, the PSI, PSII and ATP synthase complexes [2,3]. Cytochrome b6/f complexes have been well characterized in *C. reinhardtii* and we have previously described the biochemical properties of a variety of mutants altered in cytb6/f complexes [4]. Further characterization of the genetic lesions in these mutants was hampered by the lack of information on the localization and nucleotide sequences of the pet genes on the chloroplast genome of *C. reinhardtii*. Here we present a characterization of these genes in the WT strain which opens the way to an understanding of the changes in genetic expression in photosynthesis mutants altered at the level of cytb6/f complexes.

2. MATERIALS AND METHODS

Cells were grown in TAP (tris-acetate-phosphate) medium at 25°C as described by Harris [3]. Chloroplast DNA was isolated as described in Rochaix et al. [5]. Restriction enzyme digestions, DNA electrophoresis, DNA blotting were performed according to current procedures [6]. Heterologous DNA hybridizations were as described in Woessner et al. [7]. The *Hin*dIII DNA restriction fragments were cloned from an *Hin*dIII bank of WT *C. reinhardtii* in the PUN121 vector, using spinach probes or the WT restriction fragment Bg3, and then sub-cloned into bluescript vector KS- for sequencing. Dideoxy sequencing of double stranded DNA templates was performed using a sequenase (version 2) kit, according to the protocol of the supplier (US Biochemicals) with 35S-dATP (see Fig. 1 B,C for sequencing strategy). Oligonucleotide primers were synthetised using a LKB DNA-synthetiser and purified according to the protocol of the manufacturers. DNA sequences were compiled and analysed using PC GENE programmes (A. Bairoch, Department of Medical Biochemistry, University of Geneva). RNA sequencing using AMV-reverse transcriptase and a synthetic oligonucleotide as primer was done according to [8].

Correspondence address: S. Büschlen, Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, 75005 Paris, France

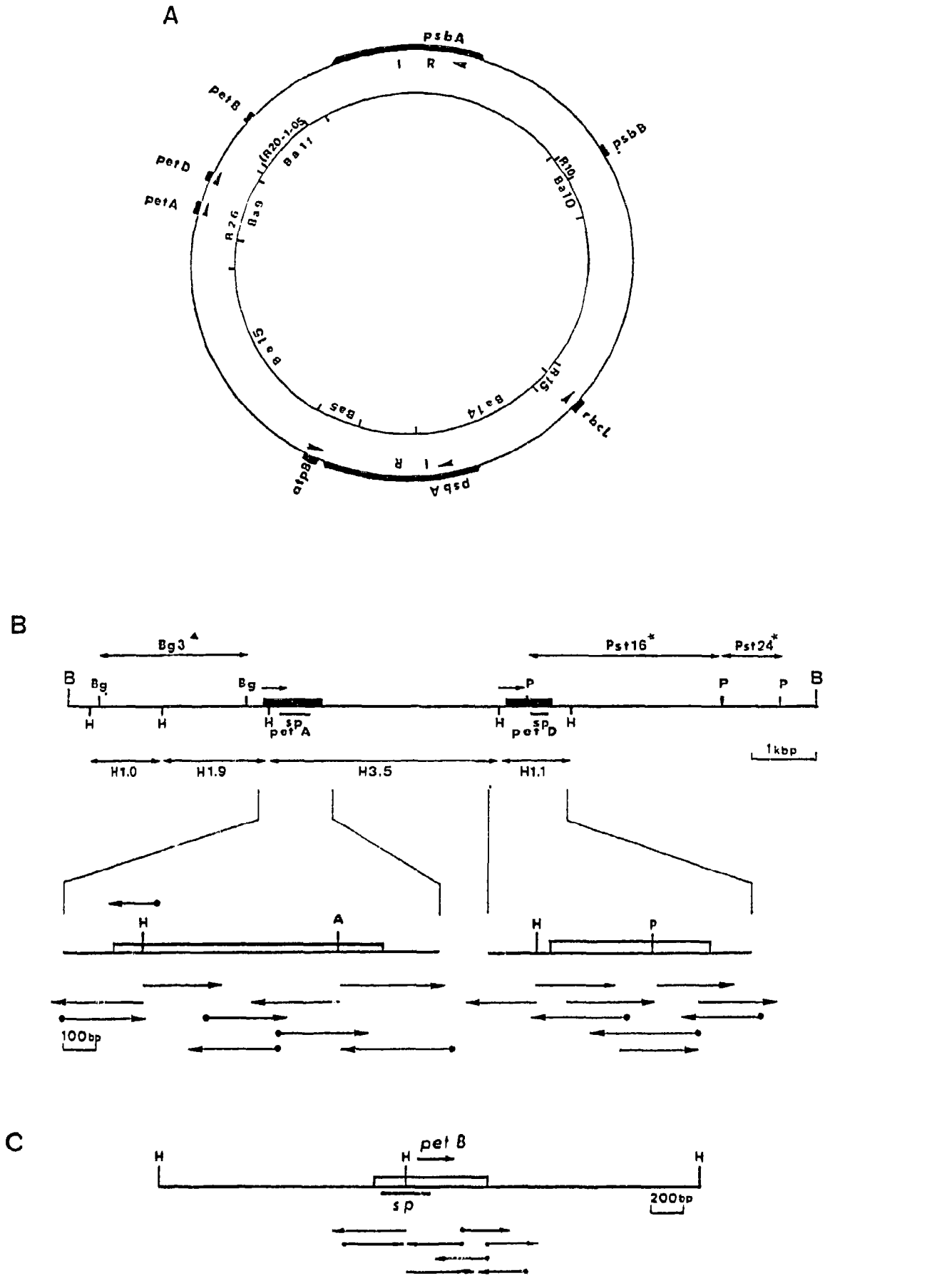
3. RESULTS AND DISCUSSION

3.1. *Localization of petA, petB and petD genes in* Chlamydomonas reinhardtii

The petA, petB, petD genes were mapped on the chloroplast genome of *C. reinhardtii* using probes from the corresponding pet genes in spinach. As indicated on Fig. 1, petB was found on the chloroplast DNA restriction fragments Ba11 (16 kb) and R20 (7.3 kb) numbered after Rochaix [9] (data not shown). Probes for petA and petD genes both hybridized to the same restriction fragments Ba9 (10.8 kb) and R26 (17.4 kb). Therefore, contrarily to what has been observed in higher plants the petB and petD genes are separated on the chloroplast genome and are not part of a psbB-psbF-petB-petD cluster [10–13]. While the localization of the psbF gene in *C. reinhardtii* is not yet known, the psbB gene is located on the restriction fragment R10, far from any of the pet genes [14].

3.2. *Cloning and sequencing of the pet genes*

In order to isolate the genes, an *Hin*dIII bank of


A
psbA
I R
petB
psbB
petD
petA
(R20-1-05
Ba11
R10
Ba10
R26
Ba9
Ba15
Bas
Ba14
IR15
rbcL
atpB
I R
psbA
B
Bg3
Pst16*
Pst24*
B
Bg
Bg
P
P
P
B
H
H
H
petA
H
petD
H
1kbp
H1.0
H1.9
H3.5
H1.1
H
A
H
P
100bp
C
pet B
H
H
H
sp
200bp

chloroplast DNA was constructed by cloning a total *Hin*dIII digest of chloroplast DNA into the pUN121 vector. This bank was then screened by hybridization with the spinach petA, petB and petD probes. The petB probe hybridized to two *Hin*dIII fragments of 1.8 and 1.5 kbp while the petA and petD probes each hybridized to single *Hin*dIII fragments of 3.5 and 1.1 kbp respectively. These fragments were subcloned into the bluescript KS- vector and sequenced by dideoxy termination chain method. While the petD gene was completely located on the 1.1 kbp *Hin*dIII fragment, the petB gene contained an internal *Hin*dIII restriction site and was therefore located on two different fragments, the N-terminal part of the gene being on the 1.5 kbp fragment and the C-terminal part of the gene being on the 1.8 kbp fragment.

The 3.5 kbp fragment that hybridized to the petA probe contained only the 287 last amino acids of the gene. To clone the adjacent *Hin*dIII fragment, encoding the 30 amino acids of the N-terminal part of the gene and corresponding to the transit peptide, we used the adjacent Bg3 fragment as a probe (see Fig. 1B) to screen the *Hin*dIII bank. It hybridized to two fragments of 1.9 and 0.9 kbp respectively, the first one being adjacent to the 3.5 kbp *Hin*dIII fragment. After subcloning in KS$^-$ we sequenced this fragment which encoded a 30 residues peptide with typical features of *C. reinhardtii* transit peptides. However, the sequence of this transit peptide was completely different from those of petA genes from higher plants. We confirmed that this transit peptide was correctly assigned to the N-terminus extension of cytf by sequencing directly the petA mRNA across the *Hin*dIII site using a synthetic oligonucleotide as primer (data not shown).

The nucleotide sequences of these three genes together with their translation in amino acid sequences are shown on Fig. 2. In contrast with what has been observed in many cases (see for instance [10–13,15]), the three pet genes are continuous. The amino acid sequences of cytb and subunit IV are highly conserved when compared with those in higher plants such as tobacco [11], with 89% and 81% of identical residues respectively, or cyanobacteria such as *Nostoc* [15], with 86% and 76% of identical residues respectively.

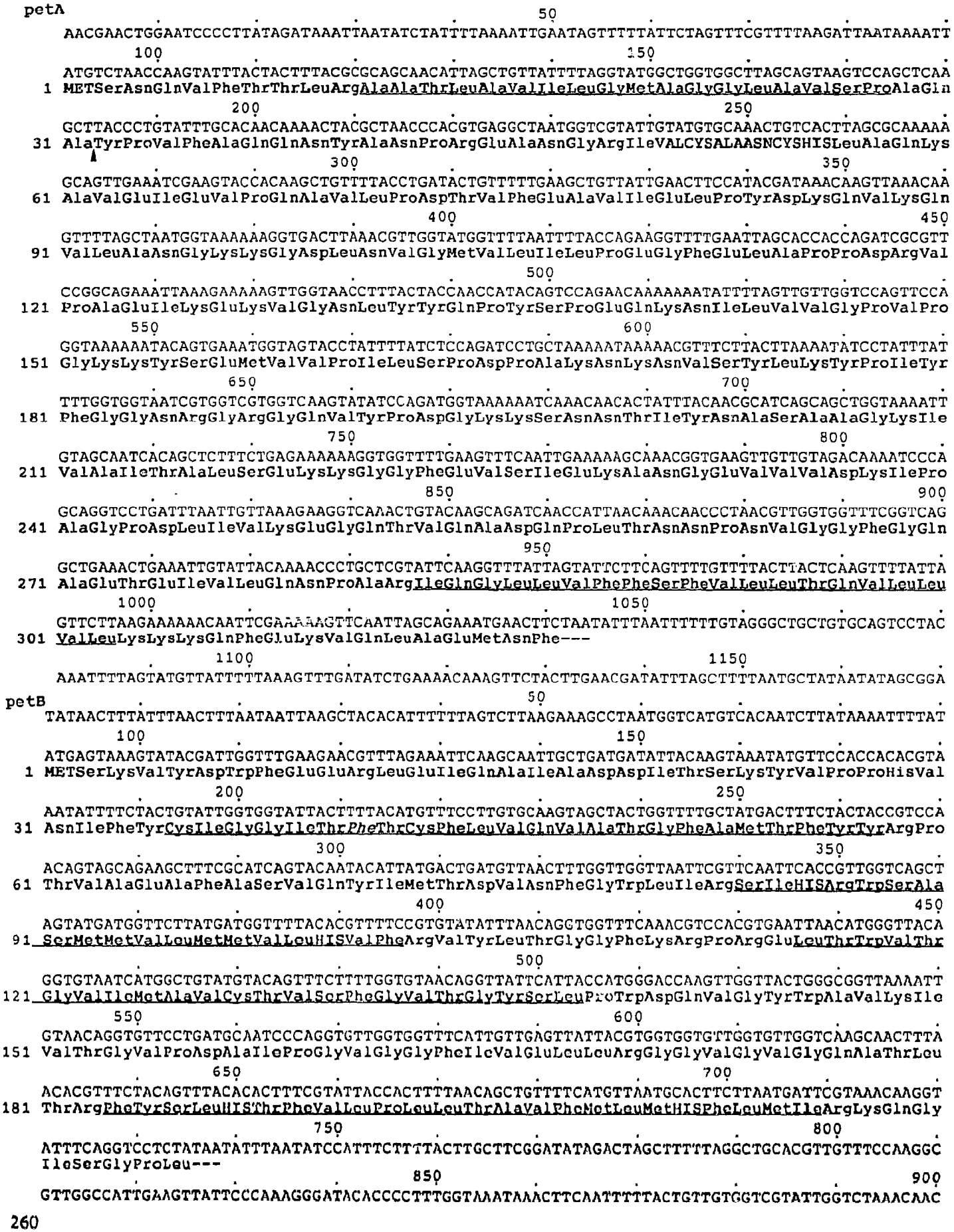
Among the amino acid changes observed in cytb6 from *C. reinhardtii*, we note that one of the sites conferring antimycin A sensitivity in yeast cytb, at Gly37 in the first transmembrane α-helix [16], is substituted for by Phe (position 41) in the present case instead of Leu in all other cytb6 sequences available in the data bank NBRF [17]. Although the antimycin A sensitivity of cyclic electron flow in higher plants has been ascribed to another site than cytb6 [18], it should be noted that cyclic electron flow is antimycin A insensitive in *C. reinhardtii* [19]. Such a Leu/Phe change is far from being conservative and is for instance responsible for the loss of funiculosin sensitivity when it occurs at position 198 in helix IV of yeast cytb [20].

Subunit IV has a 21 residues N-terminus extension, of similar length as in *Nostoc*, when compared to that in tobacco. It has two characteristics which appear specific to unicellular green algae (shared by that in *C. eugametos* [21] and in *Oocystaceae* gen. sp. [22]): a Pro-Ala-Ala-Met sequence starting at position 59, whereas subunit IV from higher plants and *Marcantia* display a Pro-Ser-Met-Ile at the same positions, and Ile instead of Lys at position 151.

Although the sequence of mature cytf is well conserved in *C. reinhardtii*, it displays less homology with its counterparts from tobacco (70%) [11] or *Nostoc* (63%) [23] than cytb6 and subunit IV. This is not surprising since most of the polypeptide chain folds in the lumen with only one α-helix spanning the membrane in the C-terminus region [24]. The transit peptide from cytf is of particular interest since it is the unique example of such a sequence for a chloroplast-encoded polypeptide in *C. reinhardtii*. Shown in Fig. 3 is a comparison of various transit peptides previously described for (A) lumenal-targeted polypeptides of nuclear origin in *C. reinhardtii* and (B) cytf from various species. Comparison of the various transit peptides in *C. reinhardtii* further confirms their mosaic structure, with a positively charged N-terminus extension observed only in nuclear-encoded polypeptides and most likely involved in the translocation across the enveloppe membrane, a hydrophobic stretch starting in positions $-25/-20$ from the final cleavage site, which is typical of lumenal polypeptides and the consensus sequence AXA for lumenal signal

←

Fig. 1. (A) Physical map of the chloroplast genome of *C. reinhardtii* (190 kpb). Outer circle shows the localization of petA, petB and petD genes with respect to atpB, rbcL, psbB and to inverted repeats containing psbA and ribosomal RNA genes (thick black bars). Arrows point to direction of transcription when it is known. Inner circle shows positions of the relevant *Eco*RI (outside) and *Bam*HI (inside) restriction fragments. (B) Restriction map of the Ba9 fragment containing the petA and petD genes. Also indicated are some restriction sites (B = *Bam*HI, Bg = *Bgl*II, H = *Hin*dIII, P = *Pst*I, A = *Acc*I). Direction of transcription is indicated by arrows. Sites of hybridization of the spinach (sp) probes are indicated below the genes (black bars). Restriction fragments are numbered after Rochaix (Bg3) [9] or Gilham for Pst (cited in [3]). Lower part of (B) shows enlargement of the two regions containing petA and petD. Sequencing strategies are illustrated by arrows with a dot where synthetic oligonucleotide primers were used, by arrows without dots when commercial primers were used in subclones originating from known restriction sites. Subclones used to sequence the petD gene were obtained by the *Exo*III deletion procedure [26]. Arrow above the petA gene represents mRNA sequencing reaction across the *Hin*dIII site. (C) Schematic map of the petB region indicating the sequencing strategy. Same symbols as in (B).


petA
AACGAACTGGAATCCCCTTATAGATAAATTAATATCTATTTTAAAATTGAATAGTTTTTATTCTAGTTTCGTTTTAAGATTAATAAAATT
ATGTCTAACCAAGTATTTACTACTTTACGCGCAGCAACATTAGCTGTTATTTTAGGTATGGCTGGTGGCTTAGCAGTAAGTCCAGCTCAA
1 METSerAsnGlnValPheThrThrLeuArgAlaAlaThrLeuAlaValIleLeuGlyMetAlaGlyGlyLeuAlaValSerProAlaGln
GCTTACCCTGTATTTGCACAACAAAACTACGCTAACCCACGTGAGGCTAATGGTCGTATTGTATGTGCAAACTGTCACTTAGCGCAAAAA
31 AlaTyrProValPheAlaGlnGlnAsnTyrAlaAsnProArgGluAlaAsnGlyArgIleVALCYSALAASNCYSHISLeuAlaGlnLys
GCAGTTGAAATCGAAGTACCACAAGCTGTTTTACCTGATACTGTTTTTGAAGCTGTTATTGAACTTCCATACGATAAACAAGTTAAACAA
61 AlaValGluIleGluValProGlnAlaValLeuProAspThrValPheGluAlaValIleGluLeuProTyrAspLysGlnValLysGln
GTTTTAGCTAATGGTAAAAAAGGTGACTTAAACGTTGGTATGGTTTTAATTTTACCAGAAGGTTTTGAATTAGCACCACCAGATCGCGTT
91 ValLeuAlaAsnGlyLysLysGlyAspLeuAsnValGlyMetValLeuIleLeuProGluGlyPheGluLeuAlaProProAspArgVal
CCGGCAGAAATTAAAGAAAAAGTTGGTAACCTTTACTACCAACCATACAGTCCAGAACAAAAAAATATTTTAGTTGTTGGTCCAGTTCCA
121 ProAlaGluIleLysGluLysValGlyAsnLeuTyrTyrGlnProTyrSerProGluGlnLysAsnIleLeuValValGlyProValPro
GGTAAAAAATACAGTGAAATGGTAGTACCTATTTTATCTCCAGATCCTGCTAAAAATAAAAACGTTTCTTACTTAAAATATCCTATTTAT
151 GlyLysLysTyrSerGluMetValValProIleLeuSerProAspProAlaLysAsnLysAsnValSerTyrLeuLysTyrProIleTyr
TTTGGTGGTAATCGTGGTCGTGGTCAAGTATATCCAGATGGTAAAAAATCAAACAACACTATTTACAACGCATCAGCAGCTGGTAAAATT
181 PheGlyGlyAsnArgGlyArgGlyGlnValTyrProAspGlyLysLysSerAsnAsnThrIleTyrAsnAlaSerAlaAlaGlyLysIle
GTAGCAATCACAGCTCTTTCTGAGAAAAAAGGTGGTTTTGAAGTTTCAATTGAAAAAGCAAACGGTGAAGTTGTTGTAGACAAAATCCCA
211 ValAlaIleThrAlaLeuSerGluLysLysGlyGlyPheGluValSerIleGluLysAlaAsnGlyGluValValValAspLysIlePro
GCAGGTCCTGATTTAATTGTTAAAGAAGGTCAAACTGTACAAGCAGATCAACCATTAACAAACAACCCTAACGTTGGTGGTTTCGGTCAG
241 AlaGlyProAspLeuIleValLysGluGlyGlnThrValGlnAlaAspGlnProLeuThrAsnAsnProAsnValGlyGlyPheGlyGln
GCTGAAACTGAAATTGTATTACAAAACCCTGCTCGTATTCAAGGTTTATTAGTATTCTTCAGTTTTGTTTTACTTACTCAAGTTTTATTA
271 AlaGluThrGluIleValLeuGlnAsnProAlaArgIleGlnGlyLeuLeuValPhePheSerPheValLeuLeuThrGlnValLeuLeu
GTTCTTAAGAAAAAACAATTCGAAAAAGTTCAATTAGCAGAAATGAACTTCTAATATTTAATTTTTTGTAGGGCTGCTGTGCAGTCCTAC
301 ValLeuLysLysLysGlnPheGluLysValGlnLeuAlaGluMetAsnPhe---
AAATTTTAGTATGTTATTTTTAAAGTTTGATATCTGAAAACAAAGTTCTACTTGAACGATATTTAGCTTTTAATGCTATAATATAGCGGA
petB
TATAACTTTATTTAACTTTAATAATTAAGCTACACATTTTTTAGTCTTAAGAAAGCCTAATGGTCATGTCACAATCTTATAAAATTTTAT
ATGAGTAAAGTATACGATTGGTTTGAAGAACGTTTAGAAATTCAAGCAATTGCTGATGATATTACAAGTAAATATGTTCCACCACACGTA
1 METSerLysValTyrAspTrpPheGluGluArgLeuGluIleGlnAlaIleAlaAspAspIleThrSerLysTyrValProProHisVal
AATATTTTCTACTGTATTGGTGGTATTACTTTTACATGTTTCCTTGTGCAAGTAGCTACTGGTTTTGCTATGACTTTCTACTACCGTCCA
31 AsnIlePheTyrCysIleGlyGlyIleThrPheThrCysPheLeuValGlnValAlaThrGlyPheAlaMetThrPheTyrTyrArgPro
ACAGTAGCAGAAGCTTTCGCATCAGTACAATACATTATGACTGATGTTAACTTTGGTTGGTTAATTCGTTCAATTCACCGTTGGTCAGCT
61 ThrValAlaGluAlaPheAlaSerValGlnTyrIleMetThrAspValAsnPheGlyTrpLeuIleArgSerIleHISArgTrpSerAla
AGTATGATGGTTCTTATGATGGTTTTACACGTTTTCCGTGTATATTTAACAGGTGGTTTCAAACGTCCACGTGAATTAACATGGGTTACA
91 SerMetMetValLeuMetMetValLeuHISValPheArgValTyrLeuThrGlyGlyPheLysArgProArgGluLeuThrTrpValThr
GGTGTAATCATGGCTGTATGTACAGTTTCTTTTGGTGTAACAGGTTATTCATTACCATGGGACCAAGTTGGTTACTGGGCGGTTAAAATT
121 GlyValIleMetAlaValCysThrValSerPheGlyValThrGlyTyrSerLeuProTrpAspGlnValGlyTyrTrpAlaValLysIle
GTAACAGGTGTTCCTGATGCAATCCCAGGTGTTGGTGGTTTCATTGTTGAGTTATTACGTGGTGGTGTTGGTGTTGGTCAAGCAACTTTA
151 ValThrGlyValProAspAlaIleProGlyValGlyGlyPheIleValGluLeuLeuArgGlyGlyValGlyValGlyGlnAlaThrLeu
ACACGTTTCTACAGTTTACACACTTTCGTATTACCACTTTTAACAGCTGTTTTCATGTTAATGCACTTCTTAATGATTCGTAAACAAGGT
181 ThrArgPheTyrSerLeuHISThrPheValLeuProLeuLeuThrAlaValPheMetLeuMetHISProLeuMetIleArgLysGlnGly
ATTTCAGGTCCTCTATAATATTTAATATCCATTTCTTTTACTTGCTTCGGATATAGACTAGCTTTTTAGGCTGCACGTTGTTTCCAAGGC
IleSerGlyProLeu---
GTTGGCCATTGAAGTTATTCCCAAAGGGATACACCCCTTTGGTAAATAAACTTCAATTTTTACTGTTGTGGTCGTATTGGTCTAAACAAC

petD

```
                                                  50
    GCCAAAATCTACTTAAATTAGATTAAAATAGTTTAAAAATGGATAGATTTAAATTAAAAAACAGAAGTTAAAATGTAATTCTGTCCCTTT
          100                                                 150
    TTACAGGGTGGTATCTCTAAAAACCAGGCTTGCCCAATCAACAATTTAAAGCTTATTTAGTTTTATTGAAAAATTAACGGATAAATAAAT
                200                                                 250
    ATGTCAGTTACTAAAAAACCTGATTTAAGCGATCCAGTTTTAAAAGCAAAATTAGCTAAAGGTATGGGTCACAACACTTACGGTGAACCT
  1 METSerValThrLysLysProAspLeuSerAspProValLeuLysAlaLysLeuAlaLysGlyMetGlyHisAsnThrTyrGlyGluPro
                      300                                                 350
    GCTTGGCCTAACGATTTATTATACATGTTCCCTGTTGTTATTTTAGGTACATTTGCATGTGTTATTGGTTTATCTGTTTTAGACCCAGCT
 31 AlaTrpProAsnAspLeuLeuTyrMetPheProValValIleLeuGlyThrPheAlaCysValIleGlyLeuSerValLeuAspProAla
                            400                                                 450
    GCTATGGGTGAGCCAGCAAACCCATTTGCTACTCCACTTGAAATTTTACCAGAATGGTATTTCTACCCTGTATTCCAAATTTTACGTGTA
 61 AlaMetGlyGluProAlaAsnProPheAlaThrProLeuGluIleLeuProGluTrpTyrPheTyrProValPheGlnIleLeuArgVal
                                  500
    GTTCCAAACAAACTTCTAGGTGTATTATTAATGGCAGCAGTACCTGCAGGCCTTATCACGGTACCGTTCATTGAAAGTATTAACAAATTC
 91 ValProAsnLysLeuLeuGlyValLeuLeuMetAlaAlaValProAlaGlyLeuIleThrValProPheIleGluSerIleAsnLysPhe
          550                                                 600
    CAAAACCCATACCGTCGTCCAATCGCTACTATCTTATTCCTTTTAGGAACTTTAGTTGCTGTTTGGTTAGGTATTGGTTCAACATTCCCT
121 GlnAsnProTyrArgArgProIleAlaThrIleLeuPheLeuLeuGlyThrLeuValAlaValTrpLeuGlyIleGlySerThrPhePro
                650                                                 700
    ATTGATATTTCTTTAACTTTAGGTTTATTCTAATCTAAAATTTTAAATTTCCCTCTAGGGTTGCAATACGATTTGCAACCTGAAGGGGAA
151 IleAspIleSerLeuThrLeuGlyLeuPhe---
```

Fig. 2. Nucleotide and deduced amino acid sequences of petA, petB and petD genes. Underlined amino acids are part of the transmembrane helix domains, the 4 histidines of cytb6 and the ValCysAlaAsnCysHis region of cytf involved in heme coordination are written in capital letters in the amino acid sequence. Underlined residues point to possible features specific of algal sequences (for details see the text). The arrow in the cytf amino acid sequence points to the cleavage site of the pre-cytf.

peptidase which is similar to that required for secretory proteins in procaryotes. The transit peptide for cytf in *C. reinhardtii* differs widely from those previously described for cytf from other sources. In particular it shows no conserved amino acid residues besides those of the cleavage site. In contrast, comparison of the transit peptides (35 residues) from five different plants available in the NBRF data bank [17] (tobacco, primrose, pea, spinach and rice, starting at the Met^{23} position for the latter) reveals conservation of 54% of the residues using Val/Leu/Ile, Lys/Arg and Asp/Glu conservative substitutions. The transit peptide of cytf in *C. reinhardtii* is particularly poor in charged residues with only one positive charge whereas its counterparts from other sources have a significantly higher net positive charge (for instance +3, +5, +4 in *Marcantia*, tobacco and *Nostoc* respectively). Its hydrophobic stretch is particularly rich in Ala and poor in Ile at variance with the characteristics of transit peptides for cytf from other eucaryotes. Changes in the relative Ala content of hydrophobic stretches have been consistently observed between eukaryotic, procaryotic and intraorganellar sorting signals [25]. One can then wonder whether pre-cytf from *C. reinhardtii* would be successfully inserted in the thylakoid membranes from higher plants.

A

```
cyt f                                MSNQVFTTLRAATLAVILGMAGGLAVSP----AQA YPVFA
cyt c     MLQLANRSVRAKAARASQSARSVSCAAAKRGADVAPLTSAIAVTASILLTTGAASASA ADLAL
p21 MALTMRNPAVKASSRVAPSSRRALRVACQAQKNETASKVGTALAASALAAAVSLSAPSA-AMA DIAGL
oee1          MALRAAQSAKAGVRAARPNRATAVVCKAKVGQAAAAAAALATAMVAGS----ANA LTFDE
oee2 MATALCNKAFAAAPVARPASRRSAVVVRASGSDVSRRAALAGFAGAAALVSSSP-----ANA AYGDS
oee3          MALASKVATRPAVASRRGAVVVRASGESRRAVLGGLLASAVAAVAPKA----ALA LTPVD
                                                                  * *
```

B

```
C.r          MSNQVFTTLRAATLAVILGMAGGLAVSP----AQA YPVFA
M.p     MQNRNFNNLIIKWAIRLISIMIIINTIFWSSI------SEA FPIYA
N.t     MQTRNAFSWLKKQITRSISVSLMIYILTRTSI------SSA YPIFA
Nos MRNASVTARLTRSVRAIVKTLLIAIATVTFYFSCDLALPQSAAA YPFWA
                                                * * *
```

Fig. 3. Comparison of lumenal-targeting transit peptides for various nuclear-encoded polypeptides in *C. reinhardtii* (taken from [27]) with transit peptide of cytf (A). Comparison of transit peptides for cytf in *C. reinhardtii* and in other organisms (B) (M.p., *Marchantia polymorpha*; N.t. *Nicotiana tobaccum*; Nos, *Nostoc* PCC 7906). Acidic residues are underlined whereas basic residues are in bold letters. *point to conserved residues.

Acknowledgements: We are greatly indebted to Dr R. Herrmann for providing the spinach cytochrome probes. We wish to thank B. Savelli for this expert technical assistance in oligonucleotide synthesis, C. de Vitry, J.-P. di Rago, P. Netter and J.-L. Popot for helpful discussions and P. Bennoun for his encouragements in the early phase of this work. This work was supported by the URA/CNRS D1187.

REFERENCES

[1] Bennoun, P. and Delepelaire, P. (1982) in: Methods in Chloroplast Molecular Biology, Edelman et al. (eds), pp. 25–38, Elsevier, Amsterdam.
[2] Boynton, J.E., Gilham, N.W., Harris, E.H., Hosler, J.P., Johnson, A.M., Jones, A.R., Randolph-Anderson, B.L., Robertson, D., Klein, T.M., Shark, K.B. and Sanford, J.C. (1988) Science 240, 1534–1538.
[3] Harris, E.H. (1989) Academic Press, San Diego, USA.
[4] Lemaire, C., Girard-Bascou, J., Wollman, F.-A. and Bennoun, P. (1986) Biochim. Biophys. Acta 851, 229–238.
[5] Rochaix, J.-D., Mayfield, S., Goldschmidt-Clermont, M. and Erickson, J. (1987) Molecular Biology of Chlamydomonas. Plant Mol. Biol.: A Practical Approach, (Schaw C.-H., ed.) pp. 253–275, IRL Press.
[6] Sambrook, K., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning; a Laboratory Manual, Cold Spring Harbor, New York.
[7] Woessner, J.P., Masson, A., Harris, E.H., Bennoun, P., Gilham, N.W. and Boynton, J.E. (1984) Plant Mol. Biol. 3, 177–190.
[8] Uzan, M., Favre, R. and Brody, E. (1988) Proc. Natl. Acad. Sci. USA 85, 8895–8899.
[9] Rochaix, J.-D. (1978) J. Mol. Biol. 126, 597–617.
[10] Heinemeyer, W., Alt, J. and Herrmann, R.G. (1984) Current Genet. 8, 543–549.
[11] Shinozaki, K., Ohme, M., Tanaka, M., Wakasugi, T., Hayashida, M., Matsubayashi, T., Zaita, N., Chunwongse, J., Obokata, J., Yamaguchi-Shinozaki, K., Ohto, C., Torazawa, K., Meng, B.Y., Sugita, M., Deno, H., Kamogashira, T., Yamada, K., Kusuda, J., Takaiwa, F., Kato, A., Tohdoh, N., Shimada, H. and Sugiura, M. (1986) EMBO J. 5, 2043–2049.
[12] Hiratsuka, J., Shimada, H., Whittier, R., Ishibashi, T., Sakamoto, M., Mori, M., Kondo, C., Honji, Y., Sun, C.R., Meng, B.Y., Li, Y.Q., Kanno, A., Nishizawa, Y., Hirai, A., Shinozaki, K. and Sugiura, M. (1989) Mol. Gen. Genet. 217, 185–194.
[13] Fukuzawa, H., Kohchi, T., Sano, T., Shirai, H., Umesono, K., Inokuchi, H., Ozeki, H. and Ohyama, K. (1988) J. Mol. Biol. 203, 333–351.
[14] Rochaix, J.-D. (1987) FEMS Microbiol. Rev. 46, 13–34.
[15] Kallas, T., Spiller, S. and Malkin, R. (1988) J. Biol. Chem. 263, 14333–14342.
[16] Di Rago, J.-P. and Colson, A.-M. (1988) J. Biol. Chem. 263, 12564–12570.
[17] Gouy, M., Gautier, C., Attimonelli, M., Lanave, C. and Di Paola, G. (1985) Comp. Appl. Biosci. 1, 167–172.
[18] Moss, D.A. and Bendall, D.S. (1984) Biochim. Biophys. Acta 767, 389–395.
[19] Bulté, L., Gans, P., Rebéillé, F. and Wollman, F.-A. (1990) Biochim. Biophys. Acta 1020, 72–80.
[20] Di Rago, J.P., Perea, J. and Colson, A.M. (1990) FEBS Lett. 263, 93–98.
[21] Turmel, M., Boulanger, J. and Bergeron, A. (1989) Nucleic Acids Res. 17, 3593.
[22] Kück, U. (1989) Mol. Gen. Genet. 218, 257–265.
[23] Kallas, T., Spiller, S. and Malkin, R. (1988) Proc. Natl. Acad. Sci. USA 85, 94–98.
[24] Hauska, G. (1986) in: Photosynthesis III Staehelin, L.A. and Arntzen C.J. (eds) pp. 496–507, Springer, Berlin.
[25] von Heijne, G., Steppuhn, J. and Herrmann, R.G. (1989) Eur. J. Biochem. 180, 535–545.
[26] Henikoff, S. (1984) Gene 28, 351–359.
[27] Franzen, L.-G., Frank, G., Zuber, H. and Rochaix, J.-D. (1989) Mol. Gen. Genet. 219, 137–144.